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Synthesis and Biological Evaluation of Pyrimidine Analogs

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Basawaraj Ballur*[1], Sangamesh Puranik[2]

[1]*Research scholar Sunrise University, Alwar, Rajasthan, India*

[2]*Research Guide Sunrise University, Alwar, Rajasthan, India*

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ABSTRACT

Their action sequences employed for synthesis of target2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazideand5-((2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)methyl)-4-phenyl-4H-1,2,4-triazole-3-thiolderivatives were illustrated in scheme1 and their analytical and physical properties are depicted in Tables. The key starting material 2 in the present study was prepared as per the literature, which will then undergo Buchwald amination with 5-bromo-2,4-dichloropyrimidine to give the target intermediate3 with superior yield. The compounds (6a-j) comprising acetohydrazide structure may exist as E/Z geometrical isomers about -C=N double bond and as cis/trans amide conformers (Figure 2). According to the literature [18-20], the compounds containing imine bonds are present in a higher percentage in-dimethyl-d6sulfoxidesolutionintheformofgeometricalEisomerabout–C=Ndoublebond.TheZ isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond. In this study, the spectral data were obtained in a dimethyl-d6sulfoxide solution and no signal belonging to the Z isomer was observed. On the other hand, thecis/transconformersofEisomerwerepresentinthedimethyl-d6sulfoxidesolutionofcompounds(6a-j). All the Synthesized compounds were characterized using spectroscopic techniques(IR, 1H NMR, 13C NMR, and LC-MS) and elemental analysis, the Analytical and Physico-chemical properties of synthesized compounds are depicted in Table 1. All existing analytical data confirms the formation of expected compounds. Formation of Compound 3 was confirmed by 1HMR spectra of the sharp singlet at δ 13.23 accountable for NH, IR Spectra showed C=O stretching band at 1728 cm-1becauseofestergroup and LC-MS. 1HNMRofcompound4showedamultipletatδ3.67-3.68and3.75-3.76 due morpholine confirms the formation. Disappearance of ethyl group and appearances of NH- NH2 signals at δ 5.74-5.95 which were absent on D2O exchange confirms scaffold 5. The lack of NH2 peak in 1HNMR, Further 13CNMR data showed a peak at δ 141.98 (HC=N-N), 144.73(C=N-N), and stretching band at 2351cm-1 of C=N of IR spectra confirms the formation of target compounds (6a-j).

INTRODUCTION

The first Bcr/Abl kinase inhibitor Imatinib [1-2] (ST1571) achieved significant clinical success. However, clinical success was soon tempered by the emergence of drug resistance [3-5]. Mutations in the kinase domain of Bcr/Abl are the major mechanism of acquired Imatinib resistance. Despite the fact that the targeted therapy era started as a hunt for selective kinase inhibitors, the aim has recently changed to find alternative strategy for discovery of new Bcr/Abl inhibitors [6] that can act on multiple targets in order to overcome the drug resistance often connected to the activation of alternative signalling pathways [7-8]. An important step forward was the approval of second generation TKIs Nilotinib (AMN107) and Dasatanib (BMS-345825), a multi targeted tyrosine kinase inhibitor, active against 14 of the 15 clinically relevant Imatinib resistant mutants [9].

The clinical success of new generation Bcr/Abl kinase inhibitors (nilotinib,Dasatanib) which are ATP-competitive agents and on basis of our previous work, according to Structure-activity relationship (SAR) analysis of the lead compound Dasatinib, in the current study we report, design, synthesis and evaluation of novel pyrimidines (Figure **1**) as potent Bcr/Abl inhibitors by well-established ADP-Glo and MTT assay method.

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder resulting from a reciprocal translocation between chromosomes 9 and 22 (t(9;22) or Philadelphia chromosome) that results in the coding of an abnormal fusion protein (BCR-ABL) with deregulated tyrosine kinase activity [1]. If not treated, CML progresses from a chronic phase (CP) to an accelerated phase (AP) and then to a blast phase (BP). Although it is a rare disease, CML was the focus of intensive investigations as a disease model against which to design tyrosine kinase inhibitors (TKIs). The understanding of the central role played by Bcr/Abl protein in the pathogenesis of CML augmented to “target therapy” [2] with synthesized small molecules to inhibit Bcr/Abl kinase activity in leukemic cells without adversely affecting the normal cell population.

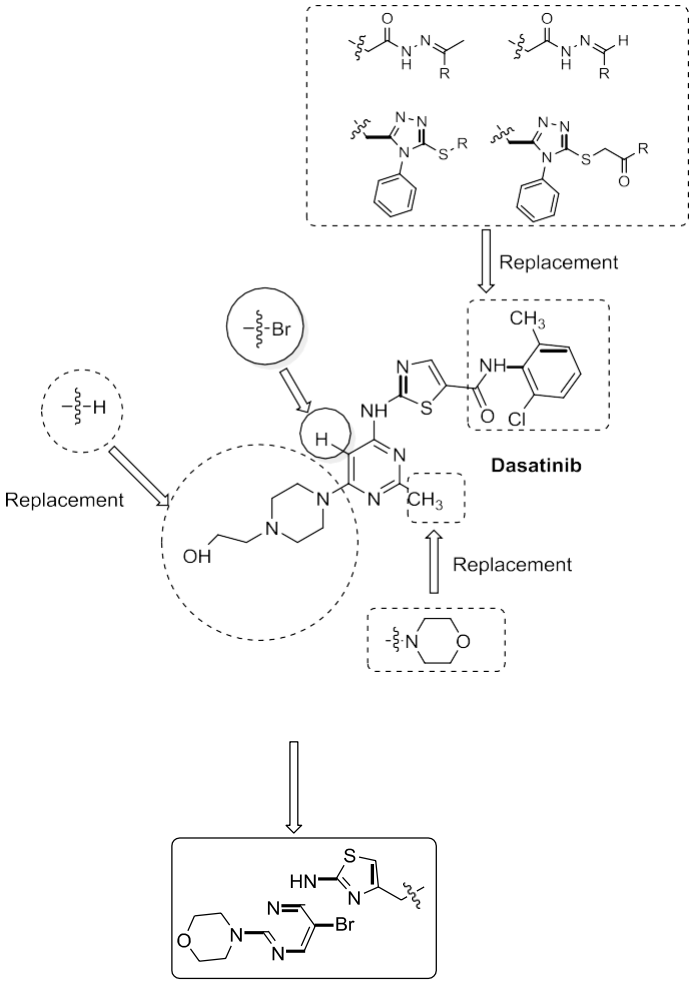
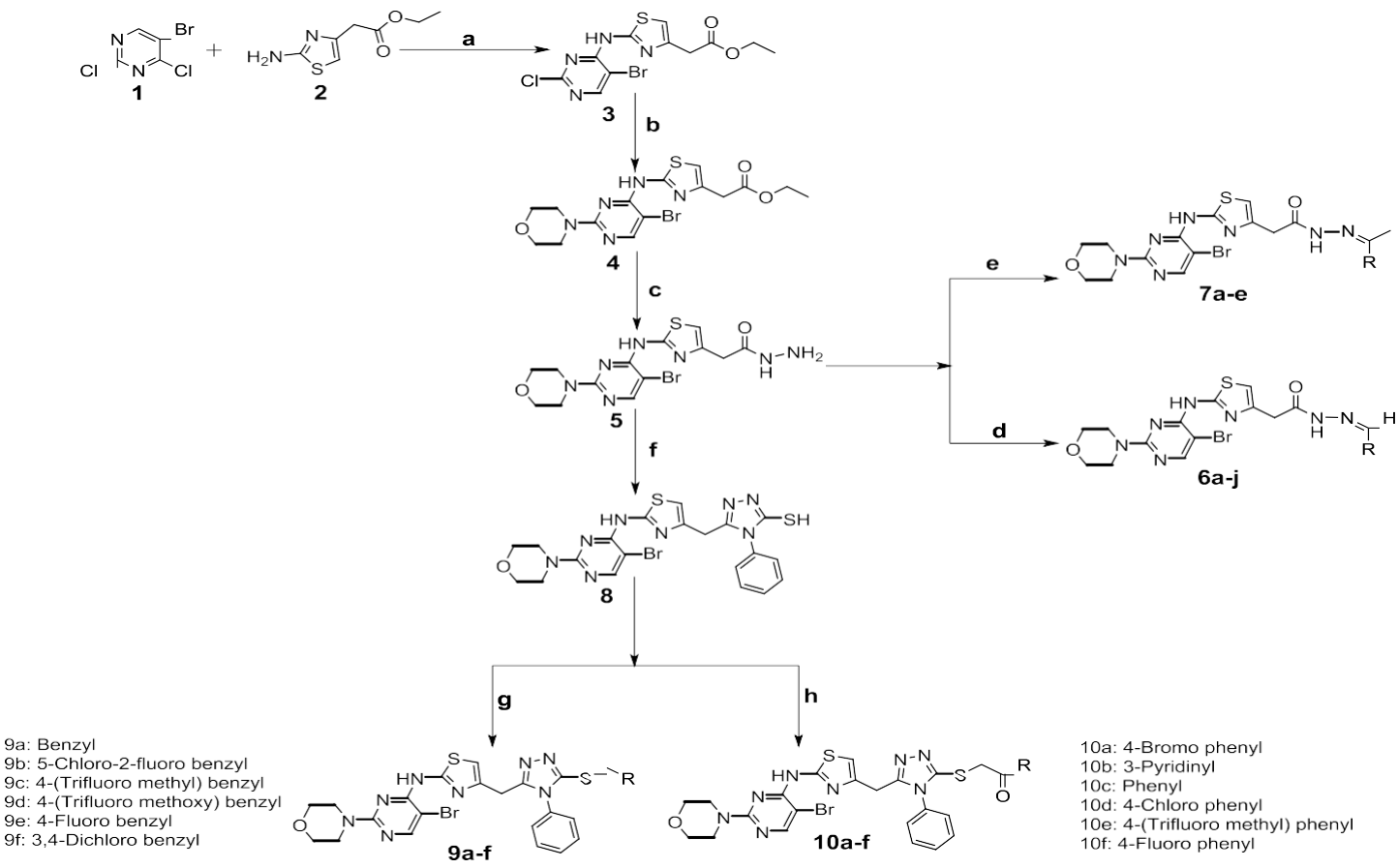


Figure 1. A design for synthesis of Dasatinib derivatives (6a-j, 7a-e, 9a-f and 10a-f)

Scheme:



Scheme 1. Synthesis pathway of clubbed 5-bromo-pyrimidine derivatives.
a) Potassiumcarbonate, 1,4 Dioxane, refluxed for 24 h. b) Ethanol, morpholine and triethylamine, refluxed for 12 h.
c) Hydrazine hydrate, ethanol, refluxed for 8 h. d) Substituted aldehyde, glacial acetic acid, ethanol, refluxed for 3 h.
e) Substituted acetophenone, glacial acetic acid, ethanol, refluxed for 3 h. f) Phenyl isothiocyanate, ethanol, sodium hydroxide, refluxed for 4 h.
g) Subsituted benzylbromides, ethanol, potassium hydroxide, 25-30 ^{o}C for 5 h. h) Subsituted phenacylbromides, ethanol, potassium hydroxide, 25-30 ^{o}C for 5 h.

Figure-1.1. Illustration of Scheme-1

The synthetic approach and steps in synthesis of target compounds:

1. In the first step, commercially available thiourea was treated with ethyl-4-bromo actoacetate in presence of ethanol medium led to the starting material **2,** which will then undergo buchwald amination with 5-bromo-2,4-dichloropyrimidine **1** to give the target intermediate **3** with superior yield. Their further condensation of compound **3** with morpholine in presence of ethanol medium led to the the formation of compound **4.**
2. The compound **4** treated with hydrazine hydrate to obtain the crucial scaffold **5.**
3. The compounds **6a-j** and **7a-e** was obtained by reacting scaffold **5** with commercially available various aldehydes and acetophenones in presence of glacial acetic acid and ethanol as medium.

1. Experimental

1.1 Synthesis of 2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl) cetohydrazide (5)

Synthesis of ethyl-2-(2-aminothiazol-4-yl)acetate (2): Charged thiourea (50g, 0.6578mol), ethyl-4-bromoactoacetate (119.19g, 0.7236mol) and ethanol (1.0L) into a RBF. The reaction mass was refluxed, stirred for 2h and completion of reaction confirmed by TLC. Ethanol removed completely and the mass quenched in to the water (500mL). Adjusted the pH to 8-8.5 using sodium bicarbonate, extracted product using ethyl acetate, concentrated the organic layer completely, triturated using n-heptane and filtered. The product was dried at 45-50°C for 5h and obtained as a yellowish to brown solid (196g, yield 80%); mp 90-95 °C.

Synthesis of ethyl-2-(2-(5-bromo-2-chloropyrimidin-4-ylamino)thiazol-4-yl) acetate (3): 5-Bromo-2,4-dichloropyrimidin **1** (50.0g, 0.2194mol), potassium carbonate(75.81g, 0,5485mol) , 1,4 dioxane (500mL) and ethyl-2-(2-aminothiazol-4-yl)acetate **2** (44.94g, 0.2413 mol) were charged into a RBF and refluxed for 24h, completion of reaction was monitored by TLC. Reaction mass was quenched into ice cold water, stirred at room temperature for 1h and the solid formed was filtered. The product was recrystallized using ethanol, dried obtained as a brown colored solid (69.6 g, yield 84%); mp 122-126 °C.

Synthesis of ethyl-2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetate (4): Ethyl-2-(2-(5-bromo-2-chloropyrimidin-4-ylamino)thiazol-4-yl)acetate **3** (60g, 0.1588mol), triethylamine (32.12g, 0.3177mol), morpholine (27.70g, 0.3177mol) and

ethanol(600mL) charged into a RBF. Reaction mass heated to reflex for 12h, completion reaction was monitored by TLC. Concentrate the ethanol completely, reaction mass was quenched into ice cold water, stirred at room temperature for 1h. The solid formed was filtered. The product was recrystallized using ethanol, dried obtained as a light Yellowish solid (59.88g, yield 88%); mp 96-98 °C.

Charged ethyl 2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetate **4** (55g, 0.1284mol), ethanol (500ml) and 98% hydrazine hydrate (50ml) into a RBF. Refluxed for 8h, completion of reaction confirmed TLC. Cool the mass to 25±5 °C, filtered and dried. The title compound was obtained as a pale brown solid **5** (47.34g, yield 89%).

M.p: 206-210 °C.,

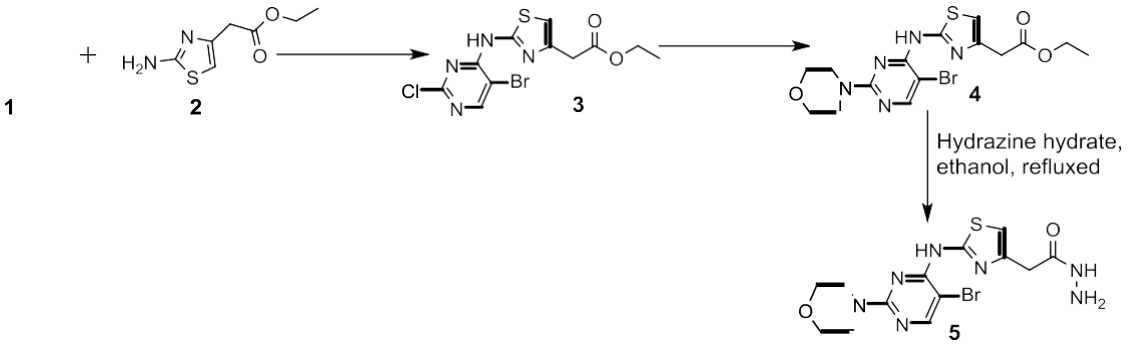
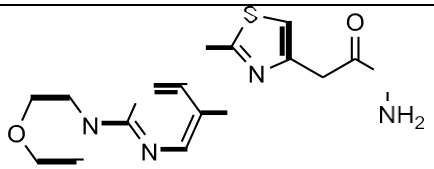


Figure-1.2: Conversion of 5-Bromo-2,4-dichloropyrimidin 1 to 2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl) acetohydrazide 5

Table-1.1: Physical and Spectral data of 2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino) thiazol-4-yl) acetohydrazide **5**

Structure					
Chemical name	Molecular formula	Molecular weight	Elemental calculated	Elemental found	TLC (Chloroform :methanol) (9:1) R_f
2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl) acetohydrazide	$C_{13}H_{16}BrN_7O_2S$	414.28	C: NA H: NA N: NA	C: NA H: NAN: NA	0.52

Infrared spectra of compound: 5

The IR spectra of compound **5** illustrates broad stretching band around 3242 cm^{-1} of N-H and strong stretching band at 1671 cm^{-1} accounting for C=O.

IR (KBr) ν_{max}/ cm^{-1} 3242 (N-H), 1671 (C=O).

^{1}H NMR spectra of compound: 5

^{1}H NMR of compound **4** showed a multiplet at δ 3.67 and 3.75 due morpholine confirms the formation, disappearance of ethyl group and appearances of NH-NH_2 signals at δ 5.74-5.95 which were absent on D_2O exchange confirms scaffold **5**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.38 (s, 2H, CH_2, thiazole-CH_2-CO), 3.65-3.67 (m, 4H, $2CH_2$ morpholine), 3.73-3.74 (m, 4H, $2CH_2$ morpholine), 5.74-5.95 (m, 3H, NH-NH_2, D_2O exchangeable), 6.78 (s, 1H, CH, thiazole), 8.13 (s,1H, CH, Pyrimidine), 9.12 (s, 1H, NH, pyrimidine-NH-thiazole, D_2O exchangeable) ppm.

LC-Mass spectra of the compound: 5

A mass spectrum of compound **5** showed a molecular ion peak m/z 414 and is consistent with its molecular weight. LC-MS (*m/z*, %) 414 (M+2, 99).

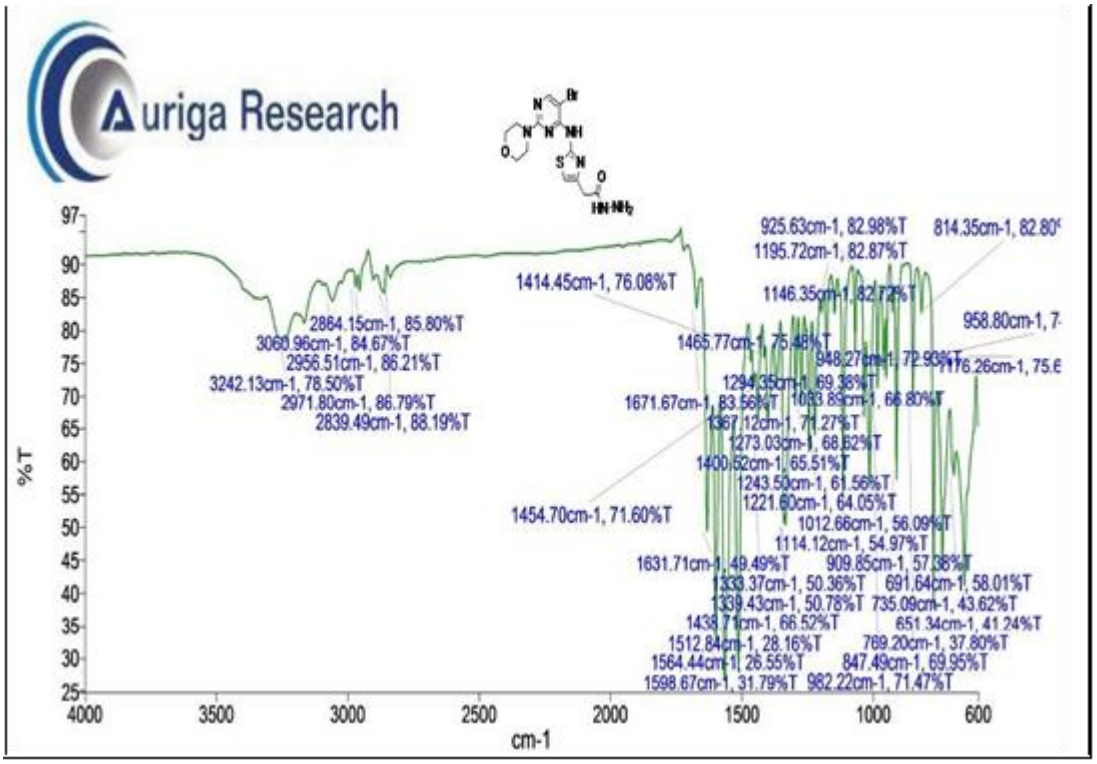


Figure-1.3: IR spectrum of compound 5

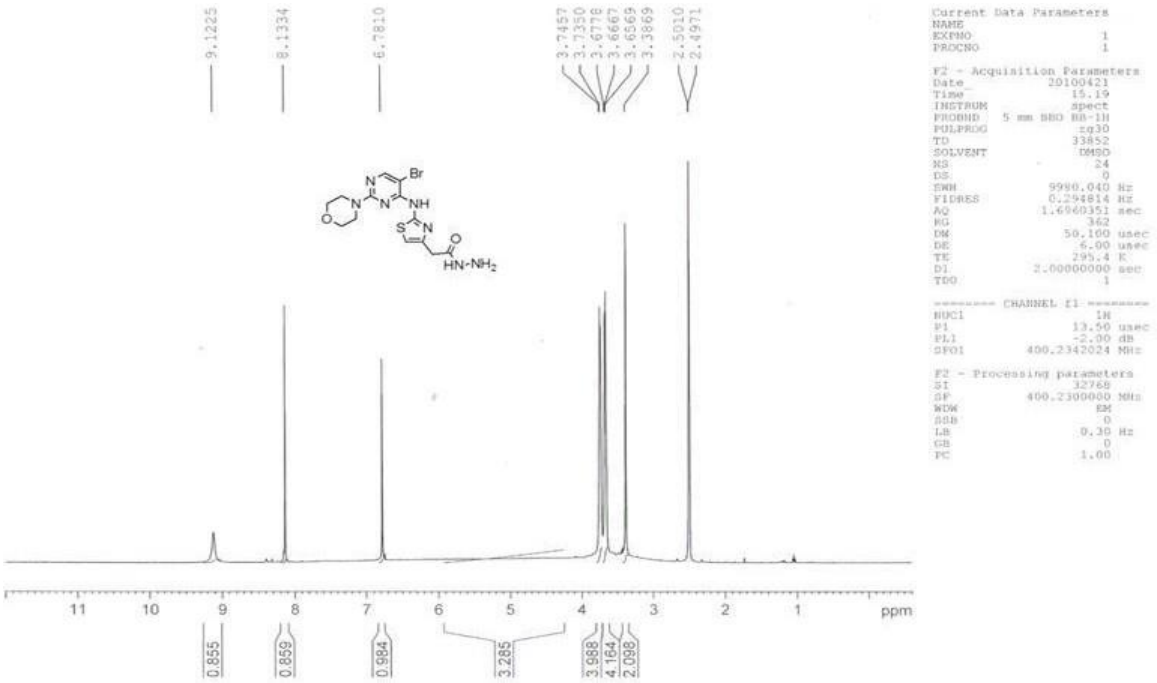


Figure-1.4: [1]HNMR of spectrum of compound 5

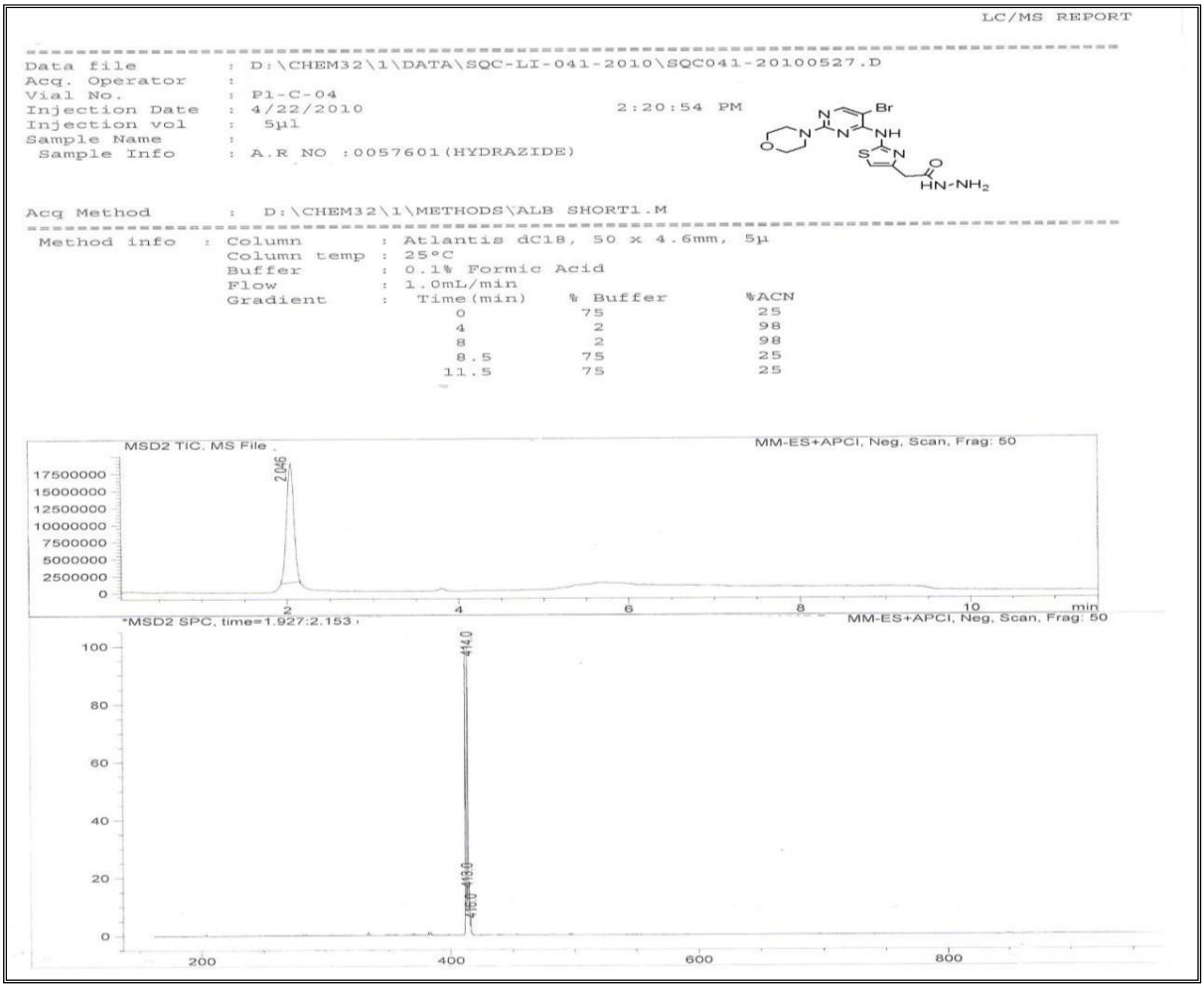
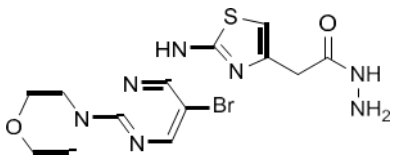


Figure-1.5: LC-Mass spectrum of compound 5

- **Chapter-7 Table-1.2:** Physical, spectral (IR) and Elemental analysis data of 2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl) acetohydrazide**5**



- General structure of compounds 5

Compound	R	Molecular Formula	M.W[a]	M.p. (°C)[b]/ crystallization solvent	Yield (%)	Position of absorption band (ν_{max}/ cm^{-1})	%Analysis of C, H, N found (calc.) [c]		
							C	H	N
5	NA	$C_{13}H_{16}BrN_7O_2S$	414.28	206-210/ ethanol	89	3242 (N-H), 1671 (C=O)	NA	NA	NA

Table-1.3: ^{1}H and ^{13}C NMR spectral data of 2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl) acetohydrazide **5**

Compound	^{1}H NMR - Chemical shift (δ) in ppm	^{13}C NMR - Chemical shift (δ) in ppm
1	^{1}H NMR (400 MHz, DMSO-*dδ*) *δ* 3.38 (s, 2H, CH_2, thiazole-CH_2-CO), 3.65-3.67 (m, 4H, $2CH_2$morpholine), 3.73-3.74 (m, 4H, $2CH_2$ morpholine), 5.74-5.95 (m, 3H, NH-NH_2, D_2O exchangeable), 6.78 (s, 1H, CH, thiazole), 8.13 (s,1H, CH, Pyrimidine), 9.12 (s, 1H, NH, pyrimidine-NH-thiazole, D_2O exchangeable) ppm.	NA

1.3.1 General procedure for the synthesis of (Z)-N'-(substituted benzylidene)-2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide (6a-j)

2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide **5** (0.01 mol), substituted aldehydes (0.01 mol) and ethanol (5V) mixture was refluxed for 3 h in the presence of few drops of glacial acetic acid. Completion of reaction was confirmed by TLC. The solvent was evaporated and residue was quenched using cold water (5V). Mass was stirred for 10 min, filtered and dried at 45-50°C. The crude solid was recrystallized using appropriate solvent systems to give target products (6a-j).

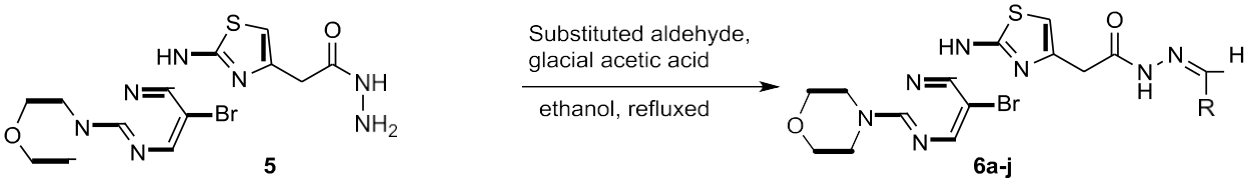


Figure-1.6: 2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide 5 to (Z)-N'-(substituted benzylidene)-2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide (6a-j)

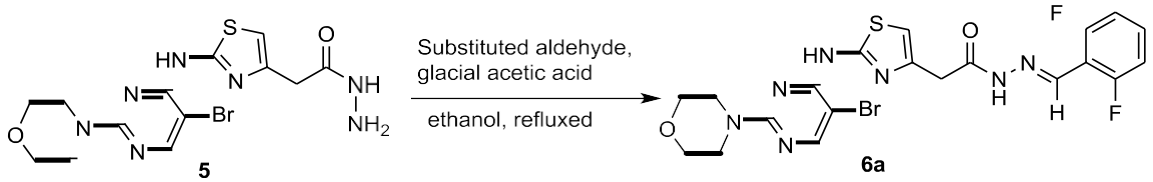


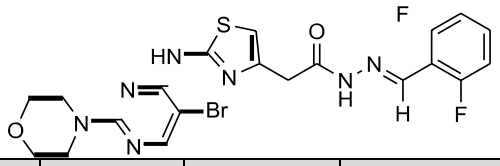
Figure-1.7: 2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide 5 to (Z)-N'-(2,6-difluorobenzylidene)-2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide 6a

1.3.1.1 Synthesis of (Z)-N'-(2,6-difluorobenzylidene)-2-(2-(5-bromo-2-morpholino pyrimidin-4-ylamino)thiazol-4-yl) cetohydrazide (6a)

2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide **5** (0.01 mol), 2,6-Difluorobenzaldehyde (0.01 mol) and ethanol (5V) mixture was refluxed for 3 h in the presence of few drops of glacial acetic acid. Completion of reaction was confirmed by TLC. The solvent was evaporated and residue was quenched using cold water (5V). Mass was stirred for 10 min, filtered and dried at 45-50°C. The crude solid was recrystallized using ethanol to give target products as an off white to pale brown solid (yield 83%).

M.p. 224-230 °C.,

Table-1.4: Physical and Spectral data of (Z)-N'-(2,6-difluorobenzylidene)-2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide **6a**

Structure					
Chemical name	Molecular formula	Molecular weight	Elemental calculated	Elemental found	TLC (Chloroform: methanol) (9:1) R_f
(Z)-N'-(2,6-difluorobenzylidene)-2-(2-(5- bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide	$C_{20}H_{18}BrF_2N_7O_2S$	538.37	C: 44.65 H: 3.38 N: 18.22	C: 44.62 H: 3.37 N: 18.21	0.63

Infrared spectra of compound: 6a

The IR spectra of compound **6a** illustrates stretching band around 3206 cm^{-1} of N-H, 2351 cm^{-1} C=N and stretching band at 1670 cm^{-1} accounting for C=O.

IR (KBr) ν_{max}/ cm^{-1} 3206 (N-H), 2351 (C=N), 1670 (C=O).

^{1}H NMR spectra of compound: 6a

Absence of NH_2 peak (δ 5.95) in ^{1}H NMR spectra confirms formation of compounds **6a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.68 (m, 4H, $2CH_2$ morpholine), 3.76 (m, 4H, $2CH_2$

morpholine), 3.99 (s, 2H, CH_2, thiazole-CH_2-CO), 6.94 (s, 1H, CH, thiazole), 7.17 (m, 2H, 2CH, 2,6-difluoro benzyl), 7.48 (m, *J* 7.2 , 1H, CH, 2,6-difluoro benzyl), 8.15 (s, 1H, CH, Pyrimidine), 8.25 (s, 1H, CH, N=CH, benzylidenimin), 10.34 (t, 1H, NH, sec amine, D_2O exchangeable), 11.62 (s, 1H, NH, sec amide, D_2O exchangeable) ppm.

^{13}C NMR spectra of compound: 6a

^{13}CNMR data showed a peak at δ 141.98 (HC=N-N) confirms formation of compound. ^{13}C NMR (100 MHz, DMSO-d_6) δ 38.6 (thiazole-CH_2-), 45.16, 66.33 (morpholine), 95.5 (pyrimidine-C_5), 104.4 (thiazole-C_3), 105.6, 111.87, 112.71, 131.86 (2,6-difluro benzyl-C_1, C_3, C_5, C_4), 141.98 (HC=N-N), 145.53 (pyrimidine-C_2), 150.8, 158.18 (thiazole-C_1, C_2), 160.14 (pyrimidine-C_6), 161.94 (2,6-difluro benzyl-C_2, C_6), 165.75 (CO, sec amide), 171.41 (pyrimidine-C_4) ppm.

LC-Mass spectra of the compound: 6a

A mass spectrum of compound **6a** showed a molecular ion peak m/z 538 and is consistent with its molecular weight. LC-MS (*m/z*, %) 538 (M+2, 99).

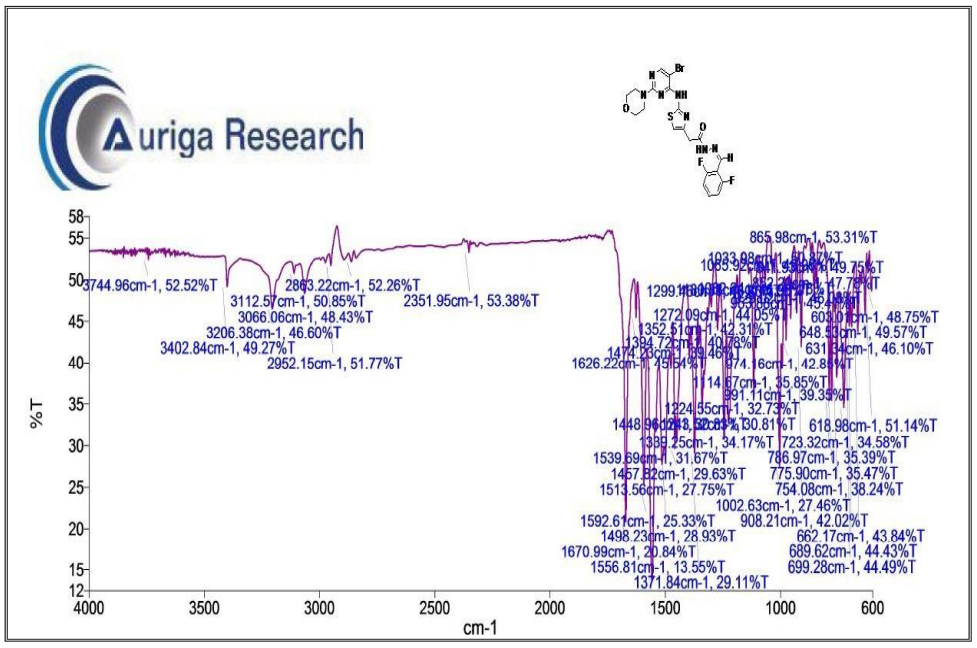


Figure-1.8: IR spectrum of compound 6a

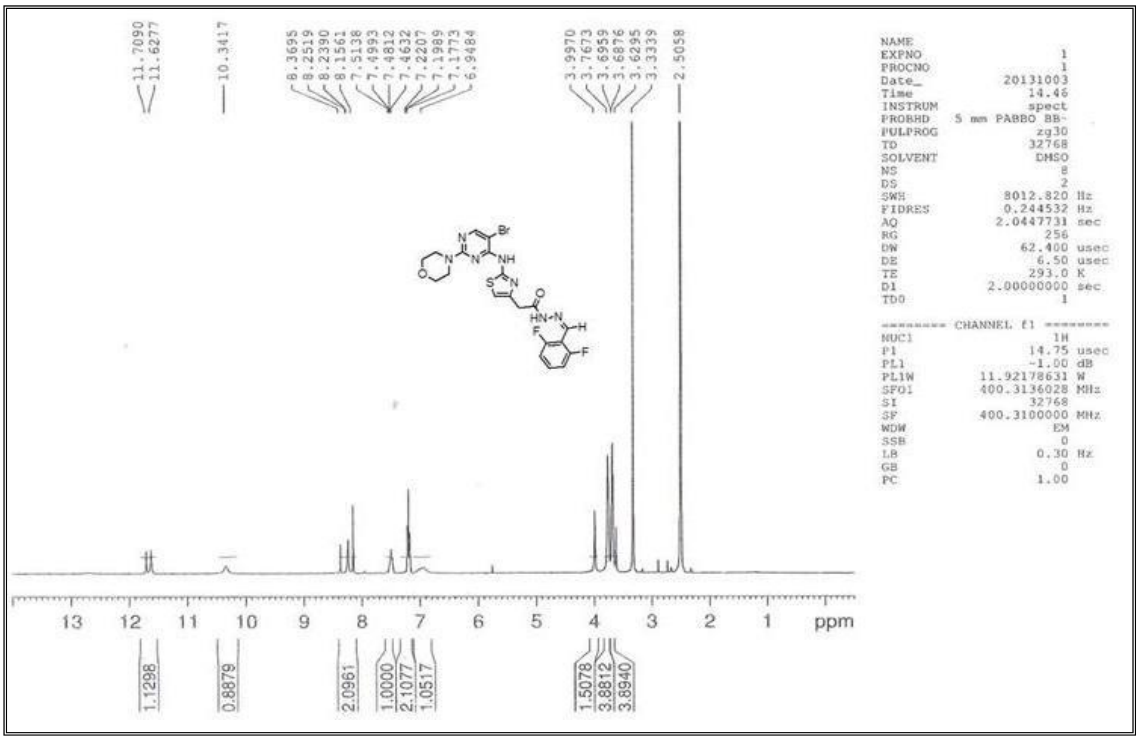


Figure-1.9: [1]HNMR of spectrum of compound 6a

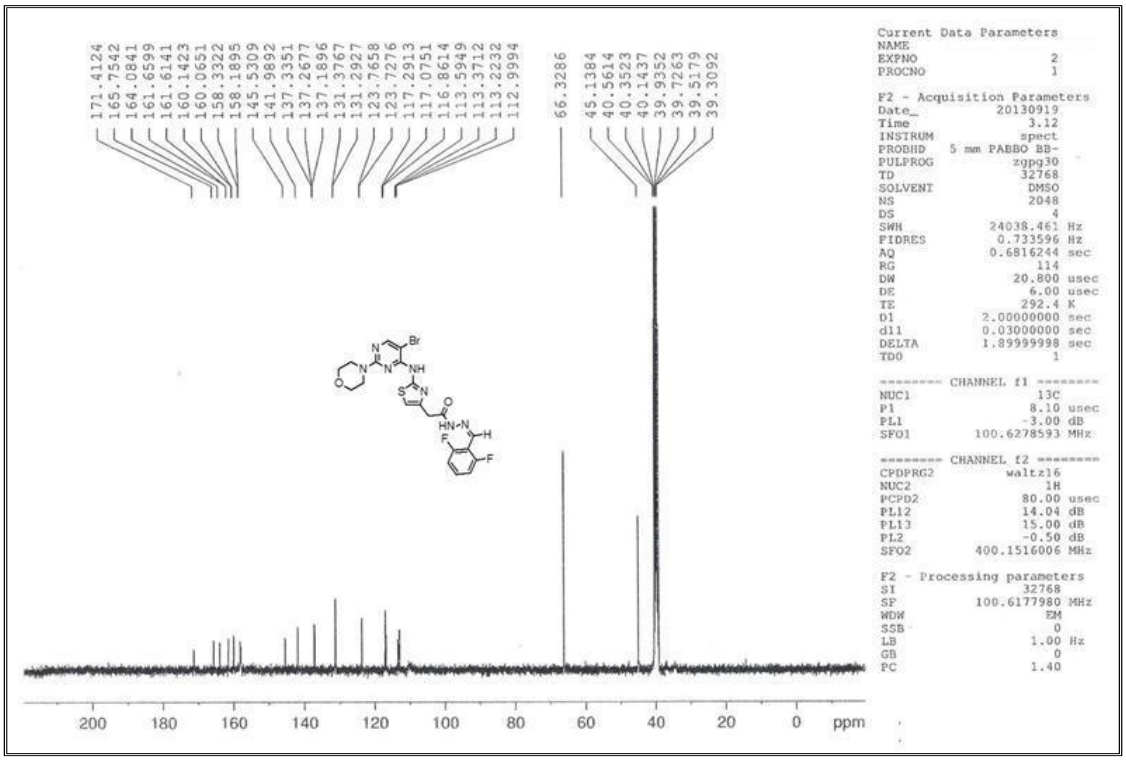


Figure-1.10: [13]CNMR of spectrum of compound 6a

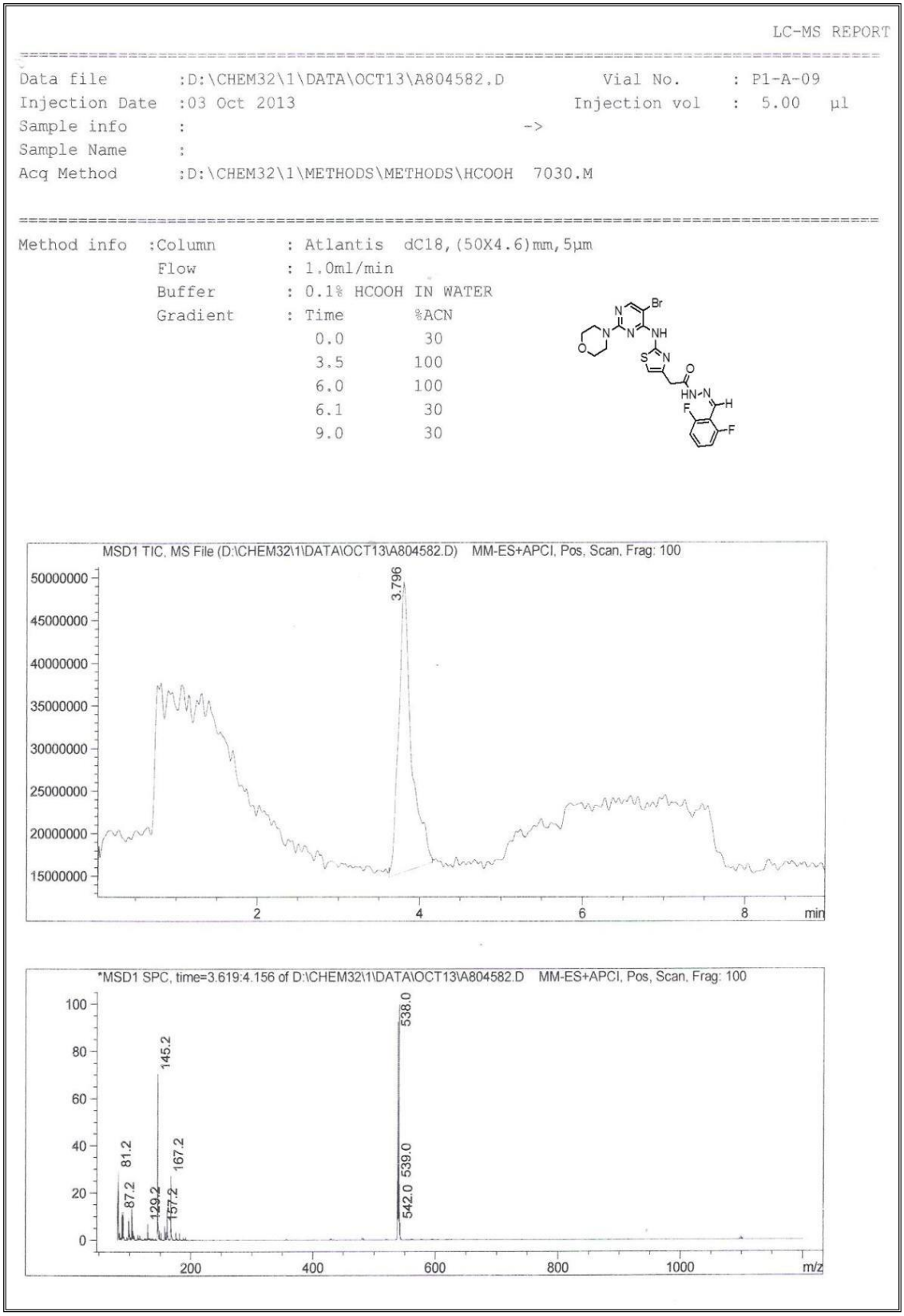
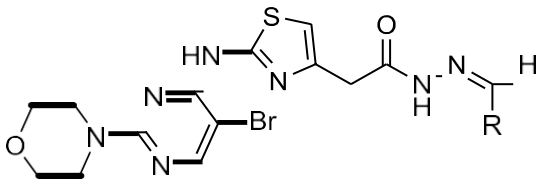


Figure-1.11: LC-Mass spectrum of compound 6a

Table-1.5: Physical, spectral (IR) and Elemental analysis data of (Z)-N'-(substituted benzylidene)-2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide



(6a-j)

General structure of compounds 6a-j

Compound	R	Molecular Formula	M.W[a]	M.p. (°C)[b]/ crystallization solvent	Yield (%)	Position of absorption band (ν_{max}/ cm^{-1})	%Analysis of C, H, N found (calc.)[c]		
							C	H	N
6a		$C_{20}H_{18}BrF_2N_7O_2S$	538.37	224-230/ ethanol	83	3206 (N-H), 2351 (C=N), 1670 (C=O)	44.62 (44.65)	3.37 (3.38)	18.21 (18.22)
6b		$C_{18}H_{18}BrN_7O_3S$	492.35	198-203/ ethanol	80	3245 (N-H), 2354 (C=N), 1672 (C=O)	43.91 (43.95)	3.68 (3.68)	19.91 (19.94)
6c		$C_{18}H_{19}BrN_8O_2S$	491.36	200-204/ ethanol	78	3235 (N-H), 2349 (C=N), 1675 (C=O)	44 (44.10)	3.9 (3.92)	22.8 (22.82)
6d		$C_{20}H_{20}BrN_7O_2S$	502.39	215-218/ ethanol	84	3214 (N-H), 2350 (C=N), 1674 (C=O)	47.81 (47.84)	4.01 (4.02)	19.52 (19.54)
6e		$C_{20}H_{19}BrClN_7O_2S$	536.83	214-218/ ethanol	81	3194 (N-H), 2276 (C=N), 1672 (C=O)	44.74 (44.75)	3.58 (3.57)	18.27 (18.26)
6f		$C_{20}H_{17}BrF_3N_7O_2S$	556.36	234-238 / ethanol	84	3201 (N-H), 2298 (C=N), 1679 (C=O)	43.18 (43.20)	3.08 (3.09)	17.62 (17.64)

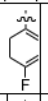
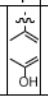
Compound	R	Molecular Formula	M.W[a]	M.p. (°C)[b]/ crystallization solvent	Yield (%)	Position of absorption band (ν_{max}/ cm^{-1})	%Analysis of C, H, N found (calc.)[c]		
							C	H	N
6g		$C_{21}H_{19}BrF_3N_7O_2S$	570.39	220-223/ ethanol	80	3193 (N-H), 2277 (C=N), 1674 (C=O)	44.23 (44.22)	3.35 (3.36)	17.18 (17.19)
6h		$C_{20}H_{19}BrFN_7O_2S$	520.38	215-218/ ethanol	79	3194 (N-H), 2276 (C=N), 1676 (C=O)	46.16 (46.18)	3.66 (3.65)	18.83 (18.84)
6i		$C_{20}H_{20}BrN_7O_3S$	518.39	217-220/ ethanol	75	3195 (N-H), 2278 (C=N), 1675 (C=O)	46.33 (46.34)	3.89 (3.89)	18.90 (18.91)
6j		$C_{21}H_{22}BrN_7O_3S$	532.42	214-217/ ethanol	81	3195 (N-H), 2278 (C=N), 1675 (C=O)	47.36 (47.37)	4.18 (4.17)	18.42 (18.42)

Table-1.6: ^{1}H and ^{13}C NMR spectral data (Z)-N'-(substituted benzylidene)-2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide **(6a-j)**

Compound	^{1}H NMR - Chemical shift (δ) inppm	^{13}C NMR - Chemical shift (δ)in ppm
6a	(400 MHz, DMSO-d_6) δ 3.68 (m, 4H, 2CH_2 morpholine), 3.76 (m, 4H, 2CH_2 morpholine), 3.99 (s, 2H, CH_2, thiazole-CH_2-CO), 6.94 (s, 1H, CH, thiazole), 7.17 (m, 2H, 2CH, 2,6-difluoro benzyl), 7.48 (m, *J* 7.2 , 1H, CH, 2,6-difluoro benzyl), 8.15 (s, 1H, CH, Pyrimidine), 8.25 (s, 1H, CH, N=CH, benzylidenimin), 10.34 (t, 1H, NH, sec amine, D_2O exchangeable), 11.62 (s, 1H, NH, sec amide, D_2O exchangeable) ppm.	100 MHz, DMSO-d_6) δ 38.6 (thiazole-CH_2-), 45.16, 66.33 (morpholine), 95.5 (pyrimidine-C_5), 104.4 (thiazole-C_3), 105.6, 111.87, 112.71, 131.86 (2,6-difluro benzyl-C_1, C_3, C_5, C_4), 141.98 (HC=N-N), 145.53 (pyrimidine-C_2), 150.8, 158.18 (thiazole-C_1, C_2), 160.14 (pyrimidine-C_6), 161.94 (2,6-difluro benzyl-C_2, C_6), 165.75 (CO, sec amide), 171.41 (pyrimidine-C_4); LC-MS (*m/z*, %) 538 (M+2, 99) ppm.
6b	(400 MHz, DMSO-d_6) δ 3.67 (m, 4H, 2CH_2 morpholine), 3.76 (m, 4H, 2CH_2 morpholine), 3.98 (s, 2H, CH_2, thiazole-CH_2-CO), 6.92 (s, 1H, CH, thiazole), 7.09 (m, 1H, CH, furan), 7.41 (m, 1H, CH, furan), 8.03 (m, 1H, CH, furan), 8.10 (s,1H, CH, Pyrimidine), 8.40 (s, 1H, CH, N=CH, benzylidenimin), 10.30 (t, 1H, NH,sec amine, D_2O exchangeable), 11.60 (s, 1H, NH, sec amide, D_2O exchangeable) ppm.	(100 MHz, DMSO-d_6) δ 38.8 (thiazole-CH_2-), 45.20, 66.35 (morpholine), 95.4 (pyrimidine-C_5), 104.5 (thiazole-C_3), 112.6, 118.90 (furan-C_3, C_2), 141.4 (HC=N-N), 144.2 (furan-C_4), 145.55 (pyrimidine-C_2), 148.34 (furan- C_1), 150.82, 158.20 (thiazole-C_1, C_2), 160.12 (pyrimidine-C_6), 165.77 (CO, sec amide), 171.40 (pyrimidine-C_4) ppm.
6c	(400 MHz, DMSO-d_6) δ 3.65 (m, 4H, 2CH_2 morpholine), 3.77 (m, 4H, 2CH_2 morpholine), 3.97 (s, 2H, CH_2, thiazole-CH_2-CO), 6.35 (m, 1H,CH, pyrrole), 6.93 (s, 1H, CH, thiazole), 7.05 (m, 1H, CH, pyrrole), 7.54 (m, 1H, CH, pyrrole), 8.20 (s, 1H, CH, Pyrimidine), 8.58 (s, 1H, CH, N=CH, benzylidenimin), 10.32 (t, 1H, NH,sec amine, D_2O exchangeable), 11.62 (s, 1H, NH, sec amide, D_2O exchangeable) ppm.	(100 MHz, DMSO-d_6) δ 38.6(thiazole-CH_2-), 45.16, 66.33 (morpholine), 95.5 (pyrimidine-C_5), 104.41(thiazole-C_3), 110.9, 119.2, 124.8, 132.71 (pyrrole-C_4, C_3, C_2, C_1), 142.5 (HC=N-N), 146.51 (pyrimidine-C_2), 150.80, 158.21 (thiazole-C_1, C_2), 160.14 (pyrimidine-C_6), 165.75 (CO, sec amide), 171.41 (pyrimidine-C_4) ppm.

	(400 MHz, DMSO-d_6) δ 3.68 (m, 4H, $2CH_2$ morpholine), 3.75 (m, 4H, $2CH_2$ morpholine), 4.02 (s, 2H, CH_2, thiazole-CH_2-CO), 6.90 (s, 1H, CH,	(100 MHz, DMSO-d_6) δ 38.62 (thiazole-CH_2-), 45.13, 66.32 (morpholine), 95.6 (pyrimidine- C_5),104.3 (thiazole-C_3), 128.8, 129.21,
6d	thiazole), 7.55-7.76 (m, 5H, 5CH, benzyl), 7.96 (s,1H, CH, Pyrimidine), 8.24 (s, 1H, CH, N=CH, benzylidenimin), 10.32 (t, 1H, NH, sec amine, D_2O exchangeable), 11.54 (s, 1H, NH, sec amide, D_2O exchangeable) ppm.	131.03, 133.6 (benzyl-C_3, C_5, C_2, C_6, C_4, C_1), 142.81 (HC=N-N), 145.55 (pyrimidine-C_2), 150.85, 158.20 (thiazole-C_1, C_2), 160.18 (pyrimidine-C_6), 165.77 (CO, sec amide), 171.42 (pyrimidine-C_4) ppm.
	(400 MHz, DMSO-d_6): δ 3.69 (m, 4H, $2CH_2$, morpholine), 3.76 (m, 4H, $2CH_2$, morpholine), 4.05 (s, 2H, CH_2, thiazole-CH_2-CO), 6.93 (s, 1H, CH, thiazole), 7.22 (t, *J* 7.96, 1H,CH,	(100 MHz, DMSO-d_6): δ 38.60 (thiazole-CH_2-), 45.13, 66.32 (morpholine), 95.5 (pyrimidine-C_5), 104.3 (thiazole-C_3), 113.59, 117.29, 123.76, 131.29, 137.20 (3-chlorobenzyl- C_1,
6e	chlorobenzyl), 7.44-7.55 (m, 3H, 3CH, 3-chlorobenzyl), 7.99 (s, 1H, CH, Pyrimidine), 8.22 (s, 1H, CH, N=CH, benzylidenimin), 10.33 (t, 1H, NH, sec amine, D_2O exchangeable), 11.59 (s, 1H, NH, sec amide, D_2O exchangeable) ppm.	C_5, C_3, C_4, C_2), 141.98 (HC=N-N), 145.53 (pyrimidine-C_2), 150.8, 158.18 (thiazole-C_1, C_2), 160.14 (pyrimidine- C_6), 161.65 (3-chlorobenzyl-C_6), 165.75(CO, sec amide), 171.41 (pyrimidine-C_4) ppm.
	(400 MHz, DMSO-d_6) δ 3.69 (m, 4H, $2CH_2$ morpholine), 3.76 (m, 4H, $2CH_2$ morpholine), 4.04 (s, 2H, CH_2, thiazole-CH_2-CO), 6.92 (s, 1H, CH, thiazole), 7.11-7.54 (m, 2H, 2CH, 2,3,4-	(100 MHz, DMSO-d_6) δ 38.60 (thiazole-CH_2-), 45.15, 66.35 (morpholine), 95.8 (pyrimidine-C_5), 104.3 (thiazole-C_3), 115.5, 120.89, 128.0, 140.20 (2,3,4-trifluorobenzyl-C_1,
6f	trifluorobenzyl), 7.98 (s, 1H, CH, Pyrimidine), 8.24 (s, 1H, CH, N=CH, benzylidenimin), 10.35 (t, 1H, NH, sec amine, D_2O exchangeable), 11.57 (s, 1H, NH, sec amide, D_2O exchangeable) ppm.	C_5, C_6, C_3), 141.98 (HC=N-N), 145.54 (pyrimidine-C_2), 145.63 (2,3,4-trifluorobenzyl-C_2), 150.81 (thiazole-C_1), 153.42 (2,3,4-trifluorobenzyl-C_4), 158.23 (thiazole-C_2), 160.14 (pyrimidine-C_6), 165.76 (CO, sec amide), 171.43 (pyrimidine-C_4) ppm.
	(400 MHz, DMSO-d_6): δ 3.68 (m, 4H, $2CH_2$, morpholine), 3.75 (m, 4H, $2CH_2$, morpholine), 4.04 (s, 2H, CH_2, thiazole-CH_2-CO), 6.92 (s, 1H, CH, thiazole), 7.19 (s, 2H, 2CH, 4-	(100 MHz, DMSO-d_6): δ 38.61 (thiazole-CH_2-), 45.14, 66.33 (morpholine), 95.51 (pyrimidine-C_5), 104.32 (thiazole-C_3), 122.85 (trifluoro carbon), 127.33, 128.64 (4-
6g	(trifluoromethyl)phenyl), 7.33 (s, 2H, 2CH, 4-(trifluoromethyl)phenyl), 7.97 (s, 1H, CH, Pyrimidine), 8.22 (s, 1H, CH, N=CH, benzylidenimin), 10.32 (t, 1H, NH, sec amine, D_2O exchangeable), 11.58 (s, 1H, NH, sec amide, D_2Oexchangeable) ppm.	(trifluoromethyl)phenyl-C_3, C_5, C_2, C_6), 134.74 (4-(trifluoromethyl)phenyl-C_4), 141.99 (HC=N-N), 145.54 (pyrimidine- C_2), 149.60 (4-(trifluoromethyl)phenyl- C_1), 150.80, 158.18 (thiazole-C_1, C_2), 160.15 (pyrimidine-C_6), 165.75 (CO, sec amide), 171.41 (pyrimidine-C_4) ppm.

	(400 MHz, DMSO-d_6): δ 3.67 (m, 4H, $2CH_2$, morpholine), 3.74 (m, 4H, $2CH_2$, morpholine), 4.02 (s, 2H, CH_2, thiazole-CH_2-CO), 6.93 (s, 1H, CH, thiazole), 7.12 (s, 2H, 2CH, 4-fluorophenyl), 7.30 (s, 2H, 2CH, 4-fluorophenyl), 7.98 (s, 1H, CH, Pyrimidine), 8.23 (s, 1H, CH, N=CH, benzylidenimin), 10.33 (t, 1H, NH, sec amine, D_2O exchangeable), 11.59 (s, 1H, NH, sec amide, D_2O exchangeable) ppm	(100 MHz, DMSO-d_6): δ 38.60 (thiazole-CH_2-), 45.13, 66.32 (morpholine), 95.50 (pyrimidine-C_5), 104.31 (thiazole-C_3), 128.8, 130.25 (4-fluorophenyl- C_3, C_5, C_2, C_6), 132.83 (4-fluorophenyl-C_1), 141.99 (HC=N-N), 145.54 (pyrimidine-C_2), 159.20 (4-fluorophenyl-C_4), 150.80, 158.18 (thiazole-C_1, C_2), 160.15 (pyrimidine-C_6), 165.75 (CO, sec amide), 171.41 (pyrimidine-C_4) ppm.
6h	(400 MHz, DMSO-d_6): δ 3.66 (m, 4H, $2CH_2$, morpholine), 3.73 (m, 4H, $2CH_2$, morpholine), 4.04 (s, 2H, CH_2, thiazole-CH_2-CO), 6.92 (s, 1H, CH, thiazole), 7.16 (s, 2H, 2CH, 4-hydroxyphenyl), 7.38 (s, 2H, 2CH, 4-hydroxyphenyl), 7.99 (s, 1H, CH, Pyrimidine), 8.22 (s, 1H, CH, N=CH, benzylidenimin), 9.10 (s, 1H, benzene-OH), 10.34 (t, 1H, NH, sec amine, D_2Oexchangeable), 11.58 (s, 1H, NH, secamide, D_2Oexchangeable) ppm.	(100 MHz, DMSO-d_6): δ 38.61 (thiazole-CH_2-), 45.12, 66.30 (morpholine), 95.51 (pyrimidine-C_5), 104.30 (thiazole-C_3), 116.0, 126.4 (4-hydroxyphenyl-$C_{3,5}$, C_1), 130.6 (4-hydroxyphenyl-$C_{2,6}$), 141.99 (HC=N-N), 145.54 (pyrimidine-C_2), 150.80, 158.18 (thiazole-C_1, C_2), 160.16 (pyrimidine-C_6), 160.80 (4-hydroxyphenyl-C_4), 165.76 (CO, sec amide), 171.42 (pyrimidine-C_4) ppm.
6j	(400 MHz, DMSO-d_6): δ 3.68 (m, 4H, $2CH_2$, morpholine), 3.72 (m, 4H, $2CH_2$, morpholine), 3.82 (s, 3H, phenyl-O-CH_3), 4.05 (s, 2H, CH_2, thiazole-CH_2-CO), 6.93 (s, 1H, CH, thiazole), 7.30 (t, 1H, CH, 3-methoxy phenyl), 7.47-7.59 (m, 3H, 3CH, 3-methoxy phenyl), 7.98 (s, 1H, CH, Pyrimidine), 8.22 (s, 1H, CH, N=CH, benzylidenimin), 10.35 (t, 1H, NH, sec amine, D_2O exchangeable), 11.57 (s, 1H, NH, sec amide, D_2O exchangeable) ppm	(100 MHz, DMSO-d_6): δ 38.60 (thiazole-CH_2-), 45.13, 66.30 (morpholine), 55.9 (CH_3, aliphatic methyl), 95.51 (pyrimidine-C_5), 104.30 (thiazole-C_3), 111.2, 116.6, 121.5 (3-methoxy phenyl-C_2, C_4, C_6), 129.8 (3-methoxy phenyl-C_5), 138.2 (3-methoxyphenyl-C_1), 141.99 (HC=N-N), 145.54 (pyrimidine-C_2), 150.80, 158.18 (thiazole-C_1, C_2), 160.16 (pyrimidine-C_6), 160.6 (3-methoxy phenyl-C_3), 165.76 (CO, sec amide), 171.40 (pyrimidine-C_4) ppm.

a. Biological protocols

1.4.1 MTT assay

The synthesized 5-bromo-pyrimidine derivatives **6a-j, is** tested in vitro for their cytotoxic properties against tumor cell lines panel consisted ofHCT116 (human colon cancer cell line), A549 (human lung cancer cell line), K562 (human chronic myeloid leukemia cell line), U937 (human acute monocytic myeloid leukemia cell line), and L02 (human normal cell line) by using MTT assay Mosmann's method. The MTT assay is based on the reduction of the soluble MTT (0.5 mg mL^{-1}, 100 µL), into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells [10].

The cells used in cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin, and streptomycin at 37 °C and humidified at 5% CO_2. Briefly cells were placed on 96-well plates at 100 µL total volume with density of 1–2.5 × 10^4 cells per ML and were allowed to adhere for 24 h before treatment with tested drugs in DMSO solution (10^{-5}, 10^{-6}, 10^{-7} mol L^{-1} final concentration). Triplicate wells were treated with media and agents. Cell viability was assayed after 96 h of continuous drug exposure with a tetrazolium compound. The supernant medium was removed, and 150 µL of DMSO solution was added to each well. The plates were gently agitated using mechanical plate mixer until the color reaction was uniform and the OD570 was determined using micro plate reader. The 50% inhibitory concentration (IC_{50}) wasdefined as the concentration that reduced the absorbance of the untreated wells by 50% of vehicle in the MTT assay. Assays were performed in triplicate on three independent experiments. The results had good reproducibility between replicate wells with standard errors below 10%.

1.4.1 Bcr/Abl inhibitory activity Assay

The Bcr/Abl inhibitory activity assay was performed using ADP-Glo™ Kinase assay kit (Promega, Catalog: V9101) according to the manufacturer's instructions. The Abl1 reaction utilizes ATP and generates ADP. Then the ADP-Glo™ reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase detection reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase reaction [11- 12].

Abl was incubated with substrates, inhibitors and ATP in a final buffer of 25 mM HEPES (pH 7.4), 10mM $MgCl_2$, 0.01% Triton X-100, 100 µ mL^{-1} BSA, 2.5 mM DTT in 384-

well plate with the total volume of 10 µL. Then the ADP-Glo^{TM} kinase assay was performed in two steps once the kinase reaction is complete. Subsequently, 5 µL ADP-Glo Reagent was added to stop the kinase reaction and deplete the unconsumed ATP. Only ADP and very low background of ATP were left. Then the mixture was incubated at room temperature for 40 min and added 10 µL of Kinase detection reagent to convert ADP to ATP and introduced luciferase and luciferin to detect ATP. At last, the mixture was incubated at room temperature for 30-60 min and measured the luminescence with a plate-reading luminometer. The signal was correlated with the amount of ATP present in the reaction and was inversely correlated with the kinase activity.

b. RESULTS AND DISCUSSION

1.5.2 Chemistry

The reaction sequences employed for synthesis of target 2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide and 5-((2-(5-bromo-2-morpholino pyrimidin-4-ylamino)thiazol-4-yl)methyl)-4-phenyl-4H-1,2,4-triazole-3-thiol derivatives were illustrated in scheme **1** and their analytical and physical properties are depicted in Tables**.** The key starting material **2** in the present study was prepared as per the literature [13], which will then undergo buchwald amination with 5-bromo-2,4-dichloropyrimidine to give the target intermediate **3** with superior yield [14].

The intermediate **4** and **5** obtained by as per the literatures [15-16], (Z)-N'-(substituted benzylidene)-2-(2-(5-bromo-2-morpholino pyrimidin-4-ylamino)thiazol-4-yl)acetohydrazide (**6a-j**) and (Z)-2-(2-(5-bromo-2-morpholinopyrimidin-4-ylamino) thiazol-4-yl)-N'-(1(substituted phenyl)ethylidene) aceto hydrazide (**7a-e**) was prepared according to the literature [17], The compounds (**6a-j)** comprising acetohydrazide structure may exist as E/Z geometrical isomers about -C=N double bond and as a cis/trans amide conformers (Figure **2**). According to the literature [18-20], the compounds containing imine bond are present in higher percentage in dimethyl-d_6 sulfoxide solution in the form of geometrical E isomer about –C=N double bond. The Z isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond. In this study, the spectral data were obtained in dimethyl-d_6 sulfoxide solution and no signal belonging to Z isomer was observed. On the other hand, the cis/trans conformers of E isomer were present in the dimethyl-d_6 sulfoxide solution of compounds (**6a-j)**.

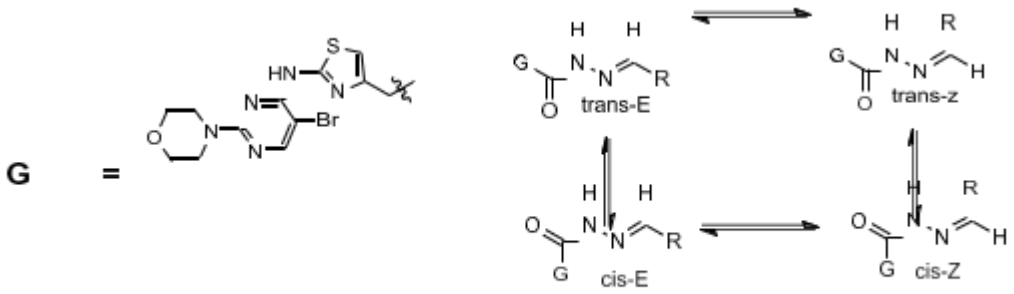


Figure 2. E/Z geometrical isomers and cis/trans confermers of 6a-j

All the Synthesized compounds were characterized using spectroscopic techniques (IR, ^{1}H NMR, ^{13}C NMR and LC-MS) and elemental analysis, the Analytical and Physico- chemical properties of synthesized compounds are depicted in Table **1**. All exiting analytical data confirms the formation expected compounds. Formation of Compound **3** confirmed by ^{1}HNMR spectra of the sharp singlet at δ 13.23 accountable for NH, IR Spectra showed C=O stretching band at 1728 cm^{-1} because of ester group and LC-MS.

^{1}H NMR of compound **4** showed a multiplet at δ 3.67-3.68 and 3.75-3.76 due morpholine confirms the formation. Disappearance of ethyl group and appearances of NH-NH_2 signals at δ 5.74-5.95 which were absent on D_2O exchange confirms scaffold **5**. The lackof NH_2 peak in ^{1}HNMR, Further ^{13}CNMR data showed a peak at δ 141.98 (HC=N-N), 144.73 (C=N-N) and stretching band at 2351cm^{-1} of C=N of IR spectra confirms the formation of target compounds **(6a-j)**.

1.5.2 Pharmacological activity and Structure Activity Relationship

1.5.2 Anticancer activity

The title compounds (6**a-j,** is evaluated for their in vitro cytotoxic activity against tumor cell lines panel consisting HCT116 (human colon cancer cell line), A549 (human lung cancer cell line), K562 (human chronic myeloid leukemia cell line), U937 (human acute monocytic myeloid leukemia cell line), and L02 (human normal cell line) by using MTT assay Mosmann's method. As most of the compounds are highly potent against K562 cells, all the synthesized compounds were evaluated for Bcr/Abl tyrosine kinase inhibitory activity by using well-established ADP-Glo assay. Dasatinib was utilized as positive control to validate in both biological evaluations.

The SAR observed with four set of title compounds are outlined in Table **7.19**. The invitro cytotoxic activity and antiproliferative studies show that the biological activity ofthese

compounds depends on (i) the nature and site of substituents on aromatic ring (ii) the length of substitution on thiazole. (iii) Study the effect of introduction phenyl-triazole All the compounds demonstrate anti-proliferation effects with IC_{50} values comparable to control standard Dasatinib.

The SAR studies indicate derivatives display moderate to high inhibitory activity against Bcr/Abl protein. In the series 6a – j, to analyse the effect of substituents on the aromatic ring R, we synthesized a number of analogs containing methoxy, halo (fluorine, bromine, chlorine), methyl, and hydroxyl groups on phenyl ring. Invitro cytotoxicity and antiproliferative inhibition results specify, among the derivatives, compound 6g with CF_3 at 4^{th} position exhibit best activity in these series with IC50 0.008 µg mL^{-1}. To further asses the significance of 4-CF_3 , we replaced these group in compound 6h with 4-F and with 2,3,4-F in compound 6f. Both these experiments resulted in partial or total loss of activity. The pharmacological evaluation of Schiff bases, 7a-f prepared with substituted ketones occasioned in additional terminal methyl group in comparison to series 6a-j. The IC50 values against tested cell lines and Bcr/Abl kinase of compound 7d confirm terminal methyl doesn't influence the biological activity.

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